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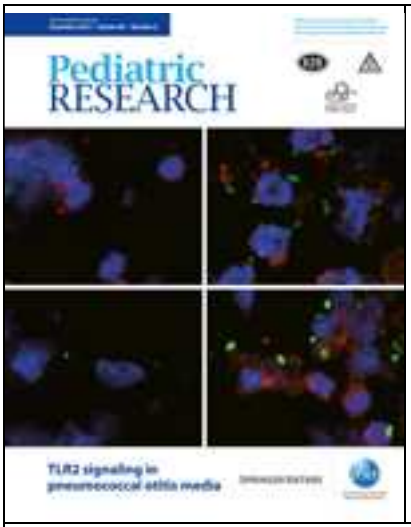

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Hemodynamic and metabolic effects of a new pediatric dobutamine formulation in hypoxic newborn pigs

Running title: New formulation of dobutamine in pigs

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† *deceased in December 2013*

Special Dedication

This manuscript is dedicated to the life of Adolf Valls-i-Soler who passed away in December 2013 after the experimental part of this work was completed.

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Category of study: translational research

ABSTRACT

Background: The aim of our study was to measure drug-related changes in hemodynamics and oxygen metabolism in response to different doses of an age-appropriate dobutamine formulation in hypoxic pigs. A secondary aim was to validate superior vena cava flow (SVCF) as a marker of cardiac index (CI) for subsequent clinical trials of this formulation in humans.

Methods: Newborn pigs (n=18) were exposed to 2h-hypoxia (10-15% oxygen) followed by reoxygenation (21-30% oxygen 4h). After 1h-reoxygenation, pigs were randomized to: control group (no treatment), dobutamine infusion at a rate of 10-15µg/kg/min or 15-20µg/kg/min. Dobutamine groups received two dobutamine doses during 30min with a 60min washout period between doses. Cardiovascular profile and oxygen metabolism were monitored. In four animals an ultrasonic perivascular flow probe was placed around superior vena cava to measure SVCF.

Results: Hypoxia significantly decreased CI, systemic-vascular-resistance and mean-arterial-blood-pressure (MABP). Dobutamine doses significantly increased heart-rate, CI and oxygen-delivery without changes in stroke-volume and MABP. Only 10-15µg/kg/min increased oxygen consumption and peripheral tissue oxygenation measured by Near-infrared-spectroscopy. A positive correlation was observed between SVCF and CI.

Conclusion: The new pediatric dobutamine formulation improved hemodynamic status, with dose-specific differences in metabolic response. SVCF may be a useful surrogate for CI in subsequent clinical trials.

INTRODUCTION

Neonatal asphyxia frequently leads to shock, myocardial dysfunction, pulmonary hypertension, poor regional perfusion, inadequate tissue oxygen delivery, impaired cerebral blood flow (CBF) autoregulation, and, potentially, end-organ injury and death (1). Importantly, in neonates surviving asphyxia there is a risk of subsequent neurological injury.

Inotropes are often used in asphyxiated infants who are exhibiting signs of cardiovascular compromise. Dobutamine, dopamine, and epinephrine are commonly used inotropes. However these drugs are currently used off label with there being little in the way of safety or efficacy data for their use in neonates. Specifically, dobutamine, a semisynthetic sympathomimetic drug, has been used off-label in newborns and children for over 20 years to enhance cardiac output (2). The importance of using licensed age-appropriate drug formulations is now recognized by regulatory agencies, clinicians and investigators (3).

In the light of the EU and USA new drug regulations on medicines for children a need exists for an age-appropriate formulation for dobutamine. Much of our current understanding of the pathophysiology of perinatal disorders has evolved from animal studies. The piglet model can be used to study perinatal hypoxia: the newborn piglet has a development similar to the 36-38 weeks human fetus (4). Moreover they share with human similar anatomic and physiologic characteristics involving the cardiovascular (with a histological appearance of the myocardium nearly identical) and other systems. These characteristics confer major advantages over models in other species; moreover its size allows the instrumentation and monitoring systemic and regional hemodynamic changes, tissue perfusion and oxygen demand. Our animal experiments were part of a larger NEO-CIRC European project (FP7_HEALTH grant agreement 282533) which studies a new age appropriate (for use in newborns in the first 72 hours of life) dobutamine formulation in order to increase availability of medicines authorized for children as well as to increase the information available on the use of medicinal products in the pediatric population.

A key factor to obtain safety and efficacy data from clinical trials of inotropes such as dobutamine is an objective measure of cardiac output (CO), and hence cardiac Index (CI, calculated as CO divided by

animal weight). CO is linked to systemic blood flow (and hence tissue perfusion), but the presence of ductal and atrial shunts in some neonates limits the usefulness of this approach and so newer ways of measuring systemic blood flow are needed. (5). Echocardiographic measurements of superior vena cava blood flow (SVCF) may provide a more reliable indication of neonatal systemic perfusion and could be used primarily in premature neonates to assess CO independent of the transitional circulation shunts. SVCF is potentially a proxy measure for CBF (6), and low SVCF has been associated with neurological injury in premature neonates.

The primary aim of the study was to measure the effects of different doses of a new pediatric formulation of dobutamine on hemodynamic and oxygen metabolism in hypoxic neonatal piglets. Our secondary aim was to validate SVCF as a marker of CI in neonatal asphyxiated piglets with normal and low CO. Furthermore we evaluated changes in biochemical parameters and cardiac histological samples after dobutamine administration.

RESULTS

The subsequent results are displayed as mean and standard deviation. The piglets were 2.0±0.9 day-old, weighing 1.7±0.2 kg with no statistically-significant differences noted in any of the baseline values between each group. Three piglets died during hypoxia and their data were excluded from the study.

Two hours of hypoxia resulted in severe metabolic acidosis (pH: 6.81-6.96; base excess (BE): -24.9-22.2 mmol/l) with a significant increase in lactate concentration (16.5-18.1 mmol/l) and normocapnia (41-44 mmHg) (Table 1). With the first dose of dobutamine (10 µg/kg/min in low to medium (L-M) group and 15 µg/kg/min in medium to high (M-H) group) pH, BE and lactate concentration demonstrated a significant improvement compared to control group (Table 1). These findings were also replicated after second dobutamine dose in both dobutamine groups (Table 1).

Superior vena cava flow (SVCF) vs. Cardiac Index (CI) correlation

32 correlations were calculated between SVCF with CI in four animals. A good positive correlation (r^2 = 0.645) was observed when SVCF was compared with CI ($p<0.0001$) (Figure 1). At baseline, the animals showed a SVCF of 75±17 ml/min/kg that decreased to 45±1 ml/kg/min during hypoxia.

Systemic hemodynamic responses

There were no baseline differences among groups in the cardiovascular parameters studied (Figure 2 and 3). Two hours of hypoxia resulted in a significant decrease in both CI (mean CI: 228-242 vs. 327-425 ml/kg/min) and systemic vascular resistance index (SVRI) (mean SVRI: 0.10-0.14 vs. 0.19-0.24 mmHg/kg/min) and severe reduction in mean arterial blood pressure (MABP) (mean MABP: 32-34 vs. 75-89 mmHg) in comparison with baseline values ($p<0.05$ in all cases), but no significant changes in stroke volume index (SVI) or heart rate (HR).

All dobutamine doses significantly increased CI with an increase in HR and a significant decrease of SVRI compared to pretreatment points but with no change in SVI (Figure 2 and 3). However, only the M-H group showed higher CI and lower SVRI than controls with a significant increase in HR, at two given dobutamine doses (15 and 20 µg/kg/min) while L-M group showed a significant increase of CI with an increase in HR only after 15 µg/kg/min dobutamine administration compared to controls.

Mean arterial blood pressure values fell below baseline values after hypoxia, during reoxygenation and after different dobutamine dose administration. MABP was maintained stable along the time without significant alteration between groups. Meanwhile, central venous pressure (CVP) of 6-8 mmHg was maintained without changes among groups or in relation to any of the doses.

Systemic oxygen metabolism and transport

At baseline there were no differences among groups in any of the systemic oxygen metabolism and transport parameters. Two hours of hypoxia produced a significant decrease in arterial oxygen content (CaO_2), oxygen delivery (OD) and oxygen consumption (VO_2) (Table 1 and Figure 4) with a significant increment of systemic fractional oxygen extraction (FTOE) ($p<0.05$ vs. baseline).

The administration of all dobutamine doses produced a significant increase in OD in comparison to controls. However, only the 10-15 µg/kg/min dobutamine doses resulted in VO_2 increment without changes in CaO_2 and FTOE.

Peripheral tissue oxygenation index (pTOI) and peripheral intravascular oxygenation (pIO2) measured by near-infrared spectroscopy (NIRS) showed a significant decrease at the end of hypoxia (pTOI: 58±5 vs. 26±9; pIO_2: -2±2 vs. -53±6). After 60 min of reoxygenation these parameters recovered partially in all

animals (pTOI: 49±5; pIO_2: -19±5). Only the L-M group showed a significant improvement of regional circulation and oxygen saturation after first (pTOI: 54±4 vs. 49±5; pIO_2: -10±4 vs. -19±5) and second (pTOI: 56±3 vs. 52±4; pIO_2: -6±3 vs. -13±2) dobutamine dose administration compared to baseline.

Biochemical and histological analysis

Baseline serum cardiac troponin T levels were 202±124 pg/ml. After 6 hours of experimental period the controls showed a non-significant increase of troponin T levels to 317±159 pg/ml. In contrast, the piglets given dobutamine groups showed similar cardiac troponin T levels compared to baseline (L-M group: 171±38 pg/ml and M-H group: 186±97 pg/ml). At the end of the experimental period serum creatine kinase (CK) activity was significantly increased in controls (121±63 U/l) in comparison with 15-20 µg/kg/min (43±6 U/l) ($p<0.05$) and 10-15 µg/kg/min (58±18 U/l) ($p=0.06$).

Necropsies at the end of the experiment revealed acute hemorrhagic foci and mild diffuse congestion in myocardium in 4 of the 6 controls. However, ventricular pathological changes were observed in only one animal in the 10-15 µg/kg/min dobutamine group and in two animals in 15-20 µg/kg/min dobutamine group.

DISCUSSION

In these newborn piglets, induction of hypoxia led to cardiovascular abnormalities such as hypotension, metabolic acidosis, decrease in CI and SVCF as well as an increase in biochemical markers such as serum troponin T and CK. We found that the new dobutamine formulation causes a significant increase in CI, HR and OD without changes in MABP or SV. Only the administration of 10-15 µg/kg/min showed a significant increase in VO_2 and pTOI and pIO_2.

SVCF can be used to assess systemic blood flow in newborn infants, of which approximately 80% goes to the brain (7). In the crucial early postnatal period SVCF may allow for a more accurate assessment of the status of systemic blood flow and response to different treatments as it is unaffected by cardiac shunting from the patent foramen ovale or ductus arteriosus (8). Although echocardiographic SVCF measurements are used in neonates, in newborn piglets it is quite difficult due to the piglet chest anatomy. To overcome this problem, we measured SVCF using a transit time ultrasound flow probe which is considered the standard reference method for cardiac output measurements in an animal model (9). In

our model, a positive correlation was observed when SVCF was compared with CI before, after hypoxia period and during dobutamine administration. Whilst it must be acknowledged that the method used to measure SVCF is very different to that used in human neonates, this study indicates that it is a biomarker, when measured accurately, reflects systemic blood flow. This is backed up by recent research showing that a new modified echocardiographic technique of measuring SVCF in human neonates correlates better with phased contract MRI measurements of SVCF.

In neonatal asphyxia, it is important to monitor CO and blood pressure because multisystem involvement includes hypotension and low CO. CO measurement using a technique of transpulmonary indicator dilution or ventricular outputs by echocardiography has been increasingly used (10). Dobutamine is a drug that primarily stimulates beta-1 receptors, leading to increase inotropic and chronotropic effects and to a lesser extent, stimulates beta-2 adrenergic receptors, leading to vasodilatation (decrease of SVRI). This combination of effects contributes to increase CO with decreased SVR (11,12). Previous animal research using neonatal animal models have observed that during the short-term dobutamine administration (10 min) the increase in CO is related to the chronotropic effects (increasing HR) (13), whereas the long-term (2 h) infusion demonstrated inotropic effects (increasing SV) (14). In our study, we found that the increase in CO after short-time dobutamine infusion was mainly due to the increased in HR rather than SV. Interestingly due to hypoxia, the piglets had high HR values prior to dobutamine administration, so although the increase in CO was found to be directly related to HR, this effect was attenuated at HR values of approximately 250 bpm, attenuating the dose-response effect. Neonates reliance on increasing CO through increasing their HR relates to a reduced ventricular diastolic compliance and an under-development of the neonatal myocyte making the heart less able to respond to volume loading (15).

The effect of dobutamine on MABP during neonatal period was variable. Consistent with other reports, our findings showed that dobutamine infusion had no significant effect on MABP (14, 16). A weak correlation between MABP and CO in preterm infants has been demonstrated (17) this is why MABP is not considered a good marker for CO. Therefore, the use of MABP as a measure of the inotropic action of dobutamine is unreliable, and direct flow measurement is needed. In fact, at short term infusions, dobutamine did not correct the low MABP seen in hypoxic piglets, although it did increase CO and SVCF.

These data are similar to that obtained by Devictor et al in 1988 in 6 full term human neonates with severe perinatal asphyxia. They demonstrated a significantly increased in CO, HR and aortic blood flow velocity after 10 micrograms/kg/min of dobutamine administration (18). The MABP increased but not significantly and SV remained unchanged (18). Therefore for effective increases in MABP and CO while caring for newborn infants recovering from perinatal asphyxia, another agent with vasopressive action such as dopamine or noradrenaline may be added to dobutamine infusion.

In our study severe hypoxia resulted in a significant decrease of CaO_2, and VO_2 with an increase of FTOE due to the decrease in OD. Moreover, the reoxygenation period resulted in a recovery of VO_2 values with a partial recovery of FTOE and OD. Dobutamine therapy has been suggested to increase tissue metabolic rate in healthy newborn lambs (19) whereas in hypoxic piglets OD increase was not accompanied by changes in VO_2 or FTOE (16). In our study, the administration of 10-15 µg/kg/min of dobutamine in hypoxic newborn piglets produced a significant increase in VO_2 probably due to the effect of dobutamine on OD (due to better CI) without changes in CaO_2 and FTOE, while higher dobutamine doses did not show significant increase of VO_2. This effect has also been previously observed in adult patients where dobutamine effects on oxygen metabolism were observed at lower doses but not at higher doses (20). To assess if these changes would have beneficial effects the infusion and experiment duration should be maintained over a prolonged period of time.

There is a great interest in the use of NIRS technology in the neonatal intensive care unit (NICU) as a continuous, non-invasive bedside monitoring technique to monitor adequacy of tissue oxygen metabolism and perfusion (21). Measurements of the renal pTOI and pIO_2 give insight into peripheral perfusion in general and into renal end-organ function (22, 23). Regional saturation changes exceeding >20% from baseline would be reason for concern and may indicate compromised perfusion. In our experiment, renal pTOI and pIO_2 measured by NIRS showed a significant decrease (> 40%) during hypoxia, suggesting a compromise in peripheral perfusion. During reoxygenation these values were partially restored and only in L-M group the administration of dobutamine doses result in an improvement of regional circulation and oxygen saturation. This effect in regional tissue oxygenation during low to medium dobutamine infusion was also observed in healthy neonatal piglets (24), not found differences at higher doses.

Limitations

This experimental piglet model used in our study attempts to mimic the clinical phenomenon of neonatal hypoxia that results in low CO, hypotension and a profound metabolic acidosis. However, as with all animal models of human disease there may be inter-species differences that limit the transferability of any results. For example, our piglet model of hypoxia does not seem to induce the hypercapnia seen with human neonates experiencing asphyxia which may influence the cardiovascular recovery. It is important to also recognize that the invasive method of measuring SVCF is very different to what is used in the clinical domain. However this is due to the constraints of the thoracic cavity.

CONCLUSION

In summary, the new pediatric dobutamine formulation improved hemodynamic status in hypoxic neonatal piglets, with dose-specific differences in metabolic response. SVCF seems to be a promising measure of CO and we will be investigating its value in subsequent longer-term clinical trials of this new formulation. The pattern of change seen in response to the new dobutamine was encouraging and justifies longer-term studies in human neonates.

The new age appropriated dobutamine formulation due to the reduction of sodium metabisulfite levels and presentation, is expected to improve safety in relation to possible adverse events and potential medication errors. So, further studies are needed to evaluate long-term multisystem organ functions and neurodevelopmental outcome.

METHODS

We obtained 18 newborn piglets (age 2-4 days, 1.7±0.2 kg) of both sexes from a local farm on the morning of the experiment. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals. The experimental protocol met European and Spanish regulations for protection of experimental animals (2010/63/UE and RD53/2013) and was approved by the Ethical Committee for Animal Welfare of the Cruces University Hospital, Spain (EU-03.BI#013_11).

Surgical preparation

The animals were sedated with intramuscular ketamine (15 mg/kg) and diazepam (2 mg/kg). Analgesia and anaesthesia were induced by intravenous (IV) fentanyl (5 micrograms/kg) and propofol (1.2 mg/kg) respectively and maintained by a continuous infusion of fentanyl (5-20 µg/kg/h) and propofol (2-3 mg/kg/h) administered through an ear vein. At the same time, the animals were paralysed using a continuous IV infusion of vecuronium bromide (3 mg/kg/h). A continuous three-lead ECG was established in all animals.

After a tracheotomy we inserted a 4.0 mm ID tracheal tube, connected to a neonatal ventilator (VIP Bird, Bird Products Corp., Palm Springs, CA) with the following initial settings: fraction of inspired oxygen (FiO_2) =0.25, respiratory frequency (f_R) =20 breaths/min, positive end-expiratory pressure (PEEP) =3 cmH_2O and positive inspiratory pressure (PIP) =10 cmH_2O. Deviations from acceptable blood gases values (PaO_2 90–110 mmHg, $PaCO_2$ 35–45 mmHg and pH 7.35–7.45) were corrected by adjusting the ventilator settings and/or by adding sodium bicarbonate as needed.

A thermodilution arterial catheter (5Fr, PiCCO Plus, Pulsion, München, Germany) was inserted into the femoral artery to monitor MABP and HR, to measure CO and to obtain arterial blood samples for blood gas analysis (P_{aO2}, P_{aCO2}, pH, Base Excess (BE), SaO_2), lactate and haemoglobin (Hb) (GEM Premier 4000, Instrumentation Laboratory Company, Lexington, MA). In addition, a 5 Fr three-lumen catheter was inserted into the internal jugular vein to inject cold saline, to monitor CVP, to maintain fluids (10% dextrose-saline at 5 ml/kg/h) and to obtain blood samples for blood gas analysis and for biochemical analysis. Finally, a 5 Fr three-lumen catheter was inserted into the femoral vein for IV infusion of dobutamine with an infusion pump (Alaris System with Guardarails, Cardinal Health, San Diego, CA). Blood temperature, measured by a thermistor at the thermodilution arterial catheter tip, was maintained at 38-39ºC using an overhead warmer along the experiment.

Correlation studies of SVCF vs. CI

To measure SVCF, a left anterior thoracotomy was performed in the first intercostal space in four animals. The superior venous cava was isolated and a non-invasive perivascular Doppler flow probe (MA3PS, Transonics, Ithaca, FL) was placed around it to continuously measure SVCF. SVCF values

were compared to the CI measured by thermodilution at different time points during experimental procedure (baseline, hypoxia, reoxygenation and dobutamine administration). Briefly, 3ml of cold saline (<8ºC) was injected into the central venous catheter. The injectate rapidly disperses volumetrically and thermally within the pulmonary and cardiac volumes. This volume of distribution is termed the intrathoracic volume. When the thermal signal reaches the thermistor-tipped in the femoral catheter, a temperature difference is detected and a dissipation curve is generated. Then the monitor use specific algorithms to determine the cardiac output by integrating the area under the curve of the arterial pressure vs. time trace.

Experimental design

Following surgery, the piglets were allowed to stabilise until baseline hemodynamic measures were stable (changes less than 10% over a 30-minute period). Preliminary arterial blood gas analysis was performed at this time to ensure that pH, PaO_2 and $PaCO_2$ values were within acceptable limits. After stabilization, hypoxia was induced (FiO_2: 0.1-0.15) by increasing the concentration of inhaled nitrogen gas for 120 min (25). At that moment animals were randomly assigned to:

1) Control group (n=6)**:** following 60 min of reoxygenation (FiO_2: 0.21- 0.30) animals were maintained without dobutamine treatment after the end of the experimental period;

2) Low to Medium dose group (L-M group) (n=6): after 60 min of reoxygenation (FiO_2: 0.21- 0.30), each animal received two doses of the new pediatric dobutamine formulation (Proveca LTD, Daresbury, Cheshire, UK) during 30 min (10 µg/kg/min and 15 µg/kg/min) with 60 min washout period between doses and after discontinuation of medication. All infusions was administered with an infusion pump, and

3) Medium to High dose group (M-H group) (n=6): after 60 min of reoxygenation (FiO_2: 0.21- 0.30), each animal received two doses of the new pediatric dobutamine formulation during 30 min (15 µg/kg/min and 20 µg/kg/min) with 60 min washout period between doses and after discontinuation of medication. The experimental design is shown in figure 5.

Dobutamine formulation

The new pediatric formulation of dobutamine HCl solution (12.5 mg/ml in 5 ml glass vials, Proveca LTD, Daresbury, Cheshire, UK) for injection is novel and investigational, containing half the quantity of antioxidant (sodium metabisulfite) compared to other marketed formulations due to concerns over the toxicity of this preservative in neonates. The manufacturing process is also novel, and uses nitrogen infusion and blanketing to limit the oxygen content the headspace of the vial, thereby limiting the rate of degradation of the dobutamine.

Measurements

At different time points (figure 5), blood samples were drawn from the femoral artery and the jugular vein to obtain blood samples to measure blood gases, base excess, lactate, and to perform biochemical analysis.

Animals were monitored for MABP, HR, CO, SVI, CVP and temperature (IntelliVue MP50, Phillips, The Netherlands) specifically before and after any intervention (figure 5). The following variables were calculated:

1- Cardiac index (CI) (ml/kg/min) = CO ÷ weight

2- Systemic vascular resistance index (SVRI) (mmHg/ml.kg^{-1}.min^{-1}) = (MABP-CVP) ÷ CI

3- Systemic arterial (venous) oxygen content ($Ca(v)O_2$) (O_2 ml/dl) = ($Sa(v)O_2$ x Hb x 1.39/100) + ($Pa(v)O_2$ x 0.003)

4- Systemic oxygen delivery (OD) (O_2 ml/kg/min) = CaO_2 x CI

5- Systemic oxygen consumption (VO_2) (O_2 ml/kg/min) = (CaO_2 – CvO_2) x CI

6- Systemic fractional oxygen extraction (FTOE) = (SaO_2 – SvO_2) ÷ SaO_2

Change in peripheral perfusion-oxygenation was assessed using a commercial continuous-wave method with a near-infrared spatially-resolved spectroscopy (NIRS) instrument (NIRO 200 Hamamatsu Photonics, Hamamatsu, Japan). Laser diodes produce light in the near-infrared range that is transmitted thorough a fibreoptic bundle. The relative absorption coefficients obtained are used to calculate the tissue

oxygenation index (TOI) in percent, that is, the ratio of oxyhaemoglobin (O_2Hb) to total haemoglobin (tHb), total haemoglobin being calculated as the sum of O_2Hb and deoxyhaemoglobin (HHb). pIO_2 and pTOI were continuously monitored. Changes in pIO_2, equivalent to the difference between O_2Hb and HHb, were used as a surrogate of changes in peripheral blood flow whereas pTOI was used to estimate venous oxygen saturation (pSvO2) (26). Due to the small size and the thin covering layers of tissue of term, the sensor was placed in the renal region (renal fossa) in order to evaluate changes in peripheral perfusion and oxygenation.

Biochemical and histological analysis

Serum troponin T (TnT) and serum creatinine kinase activity (CK) were determined at baseline and at the end of the experiment using specific Troponin T Elisa Kit (KA3319, Abnova, Tapei City, Taiwan) and creatinine kinase assay kit (ab155901, Abcam, Cambridge, MA).

At the end of the experiment animals were sacrificed through an overdose of anaesthesia (6% of sevoflurane, 1.5 mg/kg of vecuronium and 300 mg/kg of potassium chloride). Postmortem samples of left ventricle and left atrium were removed and fixed in 4% formaldehyde for histological analysis. Different areas of the heart were embedded in paraffin wax to prepare sections for light microscopy and stained for routine histological examination (hematoxylin-eosin). The histological examinations were carried out by a pathologist who was blinded to group assignments of these piglets.

Statistical analysis

Data was analysed using JMP statistical discovery software (version 8, SAS Institute Inc., North Carolina). One-way ANOVA was performed to assess time point differences in gas exchange, cardiovascular parameters and systemic oxygenation and perfusion as a function of group. Comparisons of results at all-time points were performed by two-way repeated-measures ANOVA as a function of group and time. Comparison of measured values before and after dobutamine administration was assessed by Student's paired t test. Simple linear correlation analysis was used to assess the relationship between SVCF measurements and CI measurements. A $p< 0.05$ was considered statistically significant. Values are expressed as mean ± SD.

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Table 1. Gas exchange, acid-base balance and lactate concentration in newborn piglets during hypoxia and dobutamine infusion

		Baseline	Hypoxia	DI 1st dose	DI 2nd dose	Final
pH	Control	7.32±0.02	6.81±0.08 §	6.98±0.12	7.11±0.17	7.15±0.13
	L-M	7.31±0.05	6.96±0.09 §	7.15±0.10*	7.27±0.03*	7.27±0.02
	M-H	7.36±0.05	6.91±0.08 §	7.16±0.10*	7.25±0.04*	7.26±0.07
$PaCO_2$ (mmHg)	Control	45±6	44±7	39±4	40±4	42±5
	L-M	48±10	41±4	47±10	39±5	45±4
	M-H	43±2	44±7	46±5*	47±6*	47±7
CaO_2 (ml/dl)	Control	9.6±1.8	2.5±1.6 §	8.8±2.3	8.9±1.7	9.0±1.8
	L-M	9.8±0.9	2.3±0.8 §	8.7±1.6	8.0±1.5	8.4±1.0
	M-H	9.3±2.0	1.7±0.1 §	8.3±2.2	8.0±1.3	8.0±1.4
BE (mmol/l)	Control	-3.3±2.9	-24.6±2.3 §	-20.3±4.2	-14.9±6.8	-14.8±8.6
	L-M	-2.3±2.8	-22.2±3.4 §	-12.1±2.4*	-8.4±1.2	-5.5±1 .1*
	M-H	-1.7±2.6	-24.9±1.2 §	-11.2±3.6*	-6.3±2.3*	-5.5± 3.8*
Lactate (mmol/l)	Control	1.5±0.7	16.7±3.6 §	13.3±4.0	9.6±5.4	7.8±6.0
	L-M	1.3±0.3	18.1±1.3 §	6.4±1.7*	2.9±1.4*	2.8±1.2
	M-H	1.4±0.2	16.5±0.1 §	6.2±1.9*	3.1±2.4*	2.9±3.0
Temperature (ºC)	Control	38.7±0.7	38.0±0.7	38.1±0.5	38.3±0.5	38.5±0.8
	L-M	38.7±0.5	38.1±0.4	38.7±0.5	38.2±0.5	38.3±0.6
	M-H	38.3±0.4	38.4±0.2	38.3±0.6	38.3±0.3	38.1±0.4
End tidal CO_2 (mmHg)	Control	49±3	43±13	41±5	41±5	42±6
	L-M	49±6	42±7	49±7	45±3	42±7
	M-H	48±4	51±5	50±3	47±3	44±3

(*) $p<0.05$ vs. control group (t test of paired means); (§) $p<0.05$ vs. baseline (t test of paired means). DI: dobutamine infusion; BE: base excess; CaO_2: arterial oxygen content

FIGURE LEGENDS

Figure 1. Correlation between superior vena cava flow (SVCF) and cardiac index (CI). The regression line and correlation coefficient ($r^2 = 0.645$) demonstrate a significant linear relationship ($p<0.0001$)

Figure 2. (A) Mean arterial blood pressure (MABP) and (B) heart rate (HR) in control group (white circle), low to medium dobutamine (L-M) dose group (black triangle) and medium to high dobutamine (M-H) dose group (black square). Data are expressed as mean and SD. *P<0.05 vs. Control group (one-way ANOVA), §P<0.05 vs. pretreatment (Student's paired t test) and †P<0.05 vs. Control group (two-way repeated-measures ANOVA as a function of group and time)

Figure 3. (A) Cardiac index (CI), (B) systemic vascular resistance index (SVRI) and (C) stroke volume index (SVI) in control group (white circle), low to medium dobutamine (L-M) dose group (black triangle) and medium to high dobutamine (M-H) dose group (black square). Data expressed as mean and SD. *P<0.05 vs. Control group (One-way ANOVA), §P<0.05 vs. pretreatment (Student's paired t test) and †P<0.05 vs. Control group (two-way repeated-measures ANOVA as a function of group and time)

Figure 4. (A) Oxygen delivery (OD), (B) oxygen consumption (VO_2) and (C) oxygen extraction (FTOE) in control group (white circle), low to medium dobutamine (L-M) dose group (black triangle) and medium to high dobutamine (M-H) dose group (black square). Data expressed as mean and SD. *P<0.05 vs. Control group (One-way ANOVA), §P<0.05 vs. pretreatment (Student's paired t test) and †P<0.05 vs. Control group (two-way repeated-measures ANOVA as a function of group and time)

Figure 5. Experimental protocol. The arrows represents the points at which the data were recorded (DI: dobutamine infusion; FiO_2: fraction of inspired oxygen; MABP: mean arterial blood pressure; HR: Heart rate; CVP: central venous pressure; CI: cardiac index; SVI: Stroke volume index; SVRI: Systemic vascular resistance index; CaO_2: arterial oxygen content; OD: oxygen delivery; VO_2: oxygen consumption; FTOE: systemic fractional oxygen extraction; pTOI: peripheral tissue oxygenation index; pIO_2: peripheral intravascular oxygenation; TnT: troponin T; CK: creatine kinase).

Figure 1

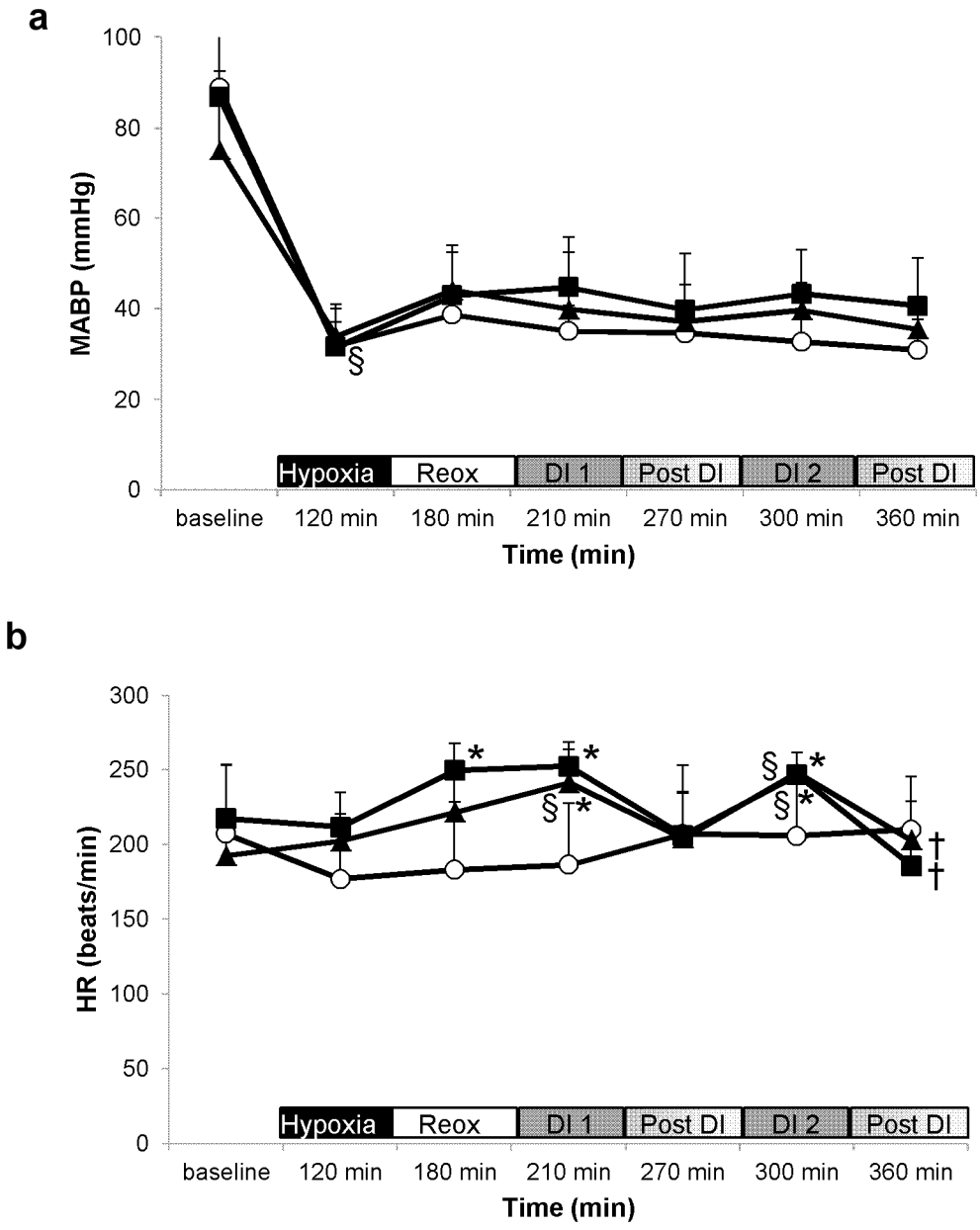

Figure 2

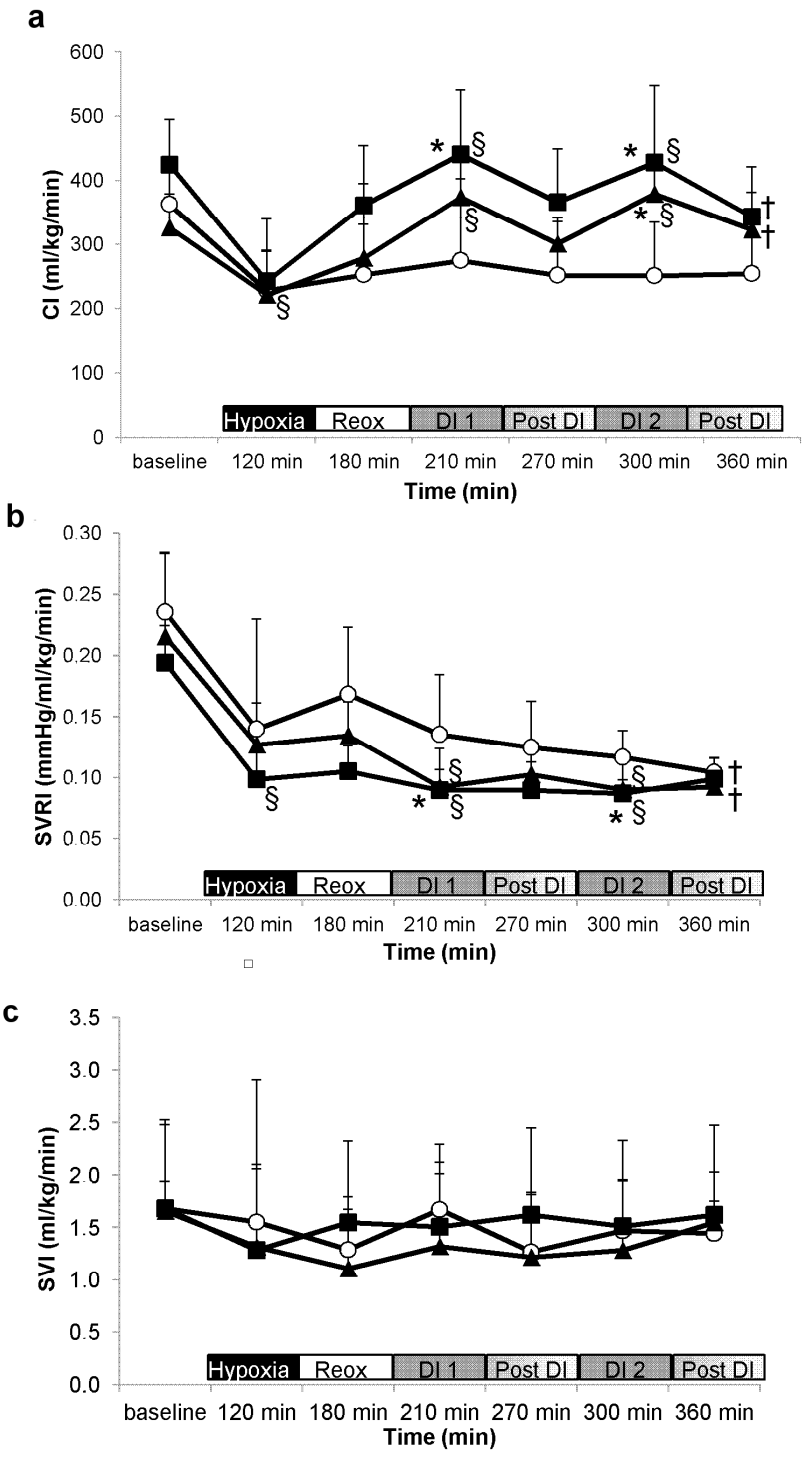

Figure 3

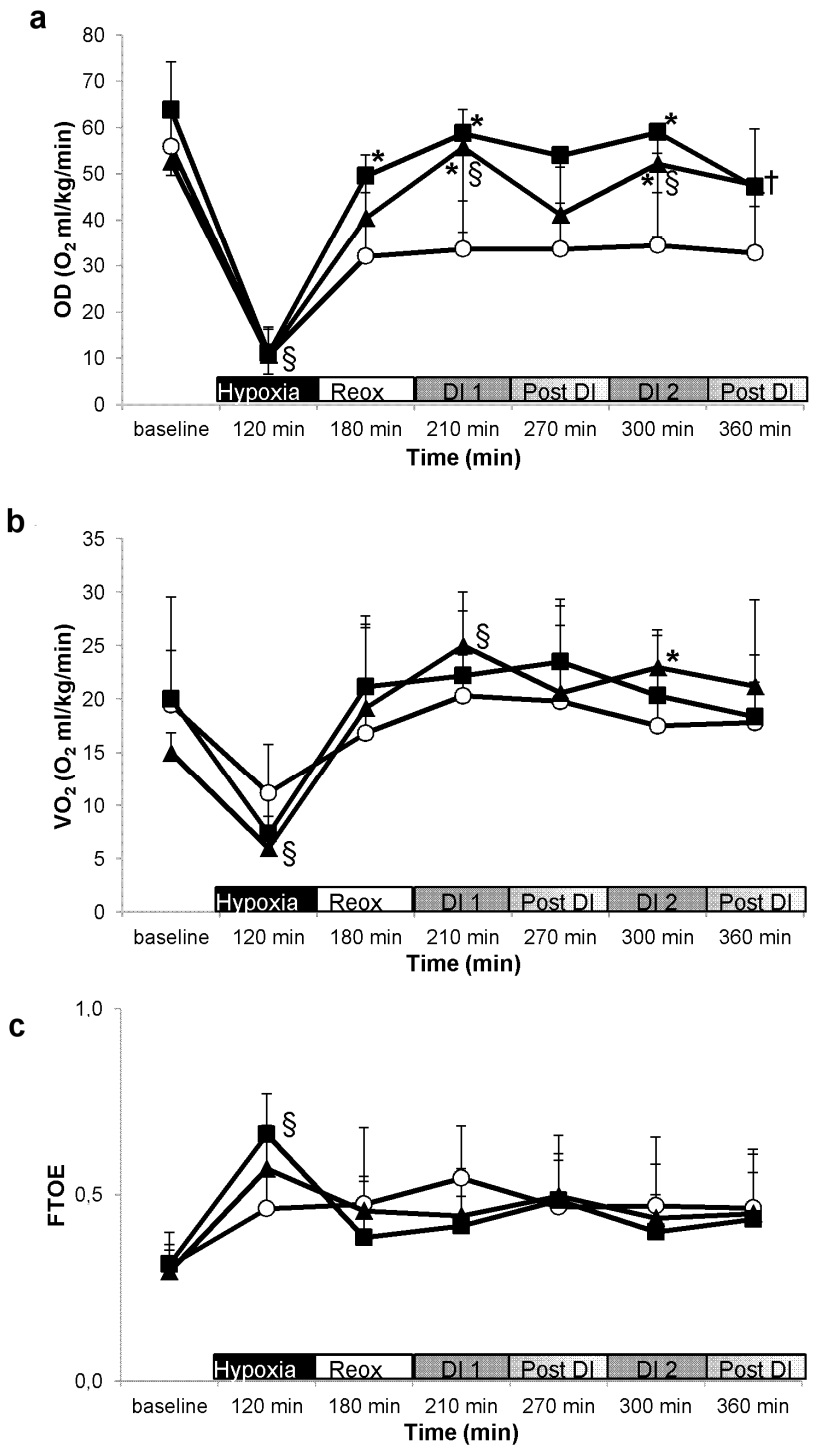

Figure 4

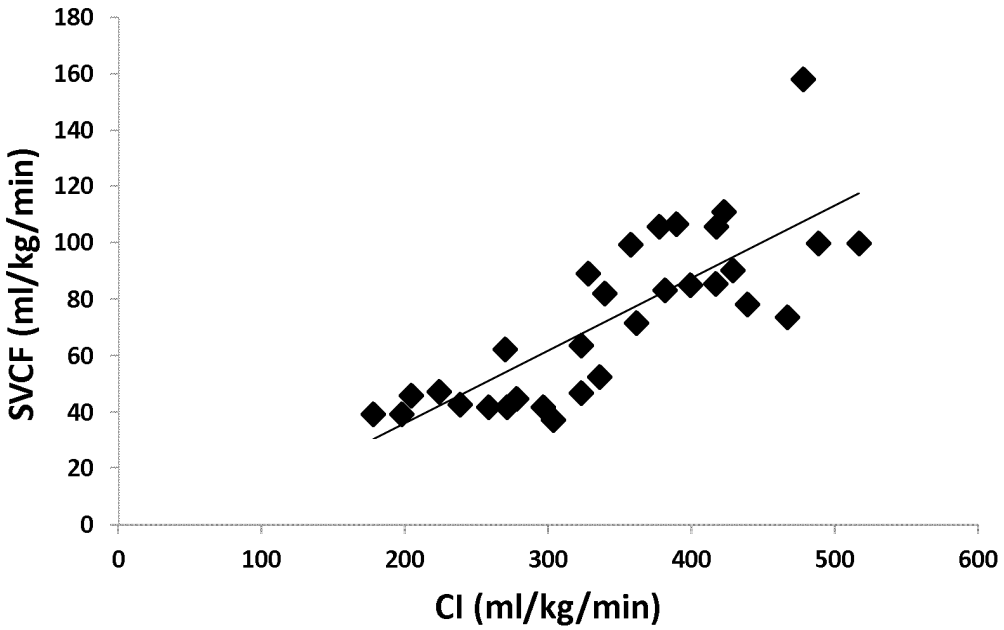

Figure 5